Fluid consumption in lithium-treated rats: Roles of stimulus novelty and context novelty

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ABSTRACT

In 5 experiments thirsty rats received an injection of lithium chloride or of saline, and their consumption of fluid was monitored at 5-min intervals for 30 min. The novelty of the fluid and the novelty of the test context was varied. In Experiment 1 a novel fluid (a sucrose solution) was offered in a novel context; in Experiment 2 the fluid was novel and the context was familiar (the home cage); in Experiment 3 the fluid was familiar and the context was novel; and in Experiment 4 both fluid and context were familiar. Lithium influenced fluid consumption in those designs that included at least one novel feature (Experiments 1, 2, and 3, but not in Experiment 4). Consumption was initially enhanced (with respect to the controls) when the context was novel, but was suppressed when the fluid was novel. In Experiment 5, the flavor was over-ingested after lithium treatment when it was presented in a short (5 min) test conducted in a novel place, but was rejected in a subsequent consumption in the home cages. It is argued that the effect of lithium depends on two factors: enhanced attention to salient cues that modifies the exploratory responses evoked by a novel context; rapid conditioning of an aversion when the fluid consumed is novel. Implications for the use of fluid consumption as an index of lithium-induced nausea are considered.

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1. Introduction

Rats given an injection of lithium chloride show a range of unconditioned responses (URs), taken to be indicative of a state of nausea and gastric illness. These include physiological changes (such as a reduction in body temperature, Batson, 1983; delayed gastric emptying, Flanagan et al., 1989; diarrhea, Nachman, 1970), and behavioral changes (e.g., a reduced level of activity, Batson, 1983; adoption of a characteristic lying-on-belly posture; Meachum and Bernstein, 1992). In addition the rats show a reluctance to consume a normally palatable novel substance. Domjan (e.g., 1977, Experiment 1) gave rats access to a saccharin solution 30 min after an injection of lithium and found a dramatic reduction in consumption (compared with that shown by control subjects given a saline injection). The effect was not a simple by-product of reduced activity, as suppression of consumption was not seen in rats given access to water rather than saccharin. This observation was taken to indicate that the effect depended on the fact that the test solution was novel. This interpretation was supported by the finding that the effect was attenuated in rats given extensive exposure to saccharin prior to the test (Domjan, 1977, Experiment 5).

Domjan (1975) described the phenomenon as poison-induced neophobia, suggesting that the state of nausea will enhance the rat's neophobic reaction to a new flavor. Earlier versions of this notion (see Rozin, 1968; Rzóska, 1953) had suggested that poisoning might increase neophobia for periods of days, but further research has confirmed that the duration of this reaction is limited to the time at which the rat is suffering the gastric illness (see Domjan, 1980, for a review). Suppressed consumption of a novel substance has thus been accepted as a useful sign of poisoning-induced nausea (e.g., Symonds and Hall, 2002) and has been promoted as a technique for assessing the ability of previously neutral cues to evoke a state of conditioned nausea (e.g., Hall and Symonds, 2006) in the development of animal models of the anticipatory nausea sometimes developed by patients undergoing chemotherapy.

In order to analyse the nature of this response more closely, Symonds and Hall (2002) gave thirsty rats access to a palatable novel solution (sucrose in their experiment) immediately after an injection of lithium. Consumption was recorded over the next 30 min, separately for each 5-min period of the test. The results (Symonds and Hall, 2002, Experiment 1) are reproduced in Fig. 1. As it can be noted, the results are in accord with those of Domjan (1977) in that the total amount of the sucrose solution consumed over the test was significantly less in the rats injected with lithium

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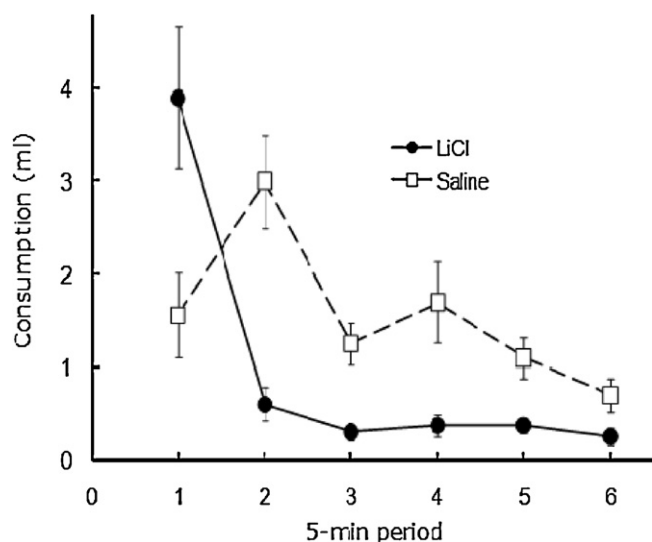E-mail address: marcial@ugr.es (M. Rodríguez).

Fig. 1. Results of Experiment 1 of Symonds and Hall (2002). Group mean scores for consumption of sucrose in each 5-min period following an injection either of LiCl or of saline. Vertical bars represent the standard error of the mean.

than in control subjects injected with saline. Notably, however, this overall suppression of consumption was a result of the behavior shown as the test progressed; suppression was not evident from the start. During the very first 5-min test period, the result was reversed, with control subjects drinking significantly less than those that had received lithium. Symonds and Hall (2002) initially suggested that such overingestion might represent an attempt by that rat to mitigate the oncoming illness, but this explanation seems unlikely to be correct. Overdrinking (antidotal thirst), as a strategy for expelling a poison through urine production, has been noted before (e.g., Smith et al., 1970), but the time course of this response is very different from what was observed by Symonds and Hall, as it occurs several hours after the injection.

The pattern of results shown in Fig. 1 is not to be expected on the basis of the hypothesis that suppression of consumption in the rats given lithium is a consequence of enhanced neophobia – on the face of things the neophobic response is likely to be at its greatest strength at the very beginning of the trial. The experiments to be described here were designed to explore possible alternatives to the enhanced-neophobia hypothesis and to elucidate the nature of the effect obtained by Symonds and Hall (2002). Our first step, after replicating the basic findings of Symonds and Hall in our Experiment 1, was to investigate the effects of lithium on consumption of a novel substance in a procedure more like that used by Domjan (1977). Domjan's procedure differed from that of Symonds and Hall in two main ways: first, there was a longer delay between the injection and the test; second, the test was given in the familiar home cage rather than in a novel context. We decided, in Experiment 2, to concentrate on this second factor given previous observations (Sjödén and Archer, 1981) suggesting that the rat's neophobic response can be modified by the novelty of the context. Thus, in Experiment 1 we measured the ingestion of a novel sucrose solution in a novel place, and in Experiment 2 the ingestion of the novel sucrose in a familiar place. In each we included detailed measurement (at 5-min intervals) of the consumption over the test period.

To anticipate, we found that the initial overconsumption in lithium-injected rats was not obtained when testing occurred in a familiar context. In order to investigate the roles of stimulus and context novelty further, in Experiments 3 and 4 we also assessed ingestion in a novel and in a familiar place, but used a familiar substance (water) as the test fluid. Thus, Experiment 3 assessed the

influence that the novelty of the context has on ingestion of a familiar fluid in poisoned rats, and Experiment 4 observed this reaction in the familiar home cages. The use of the Symonds and Hall's procedure under these four conditions, permitted us to identify the critical factors that were responsible for the differing patterns of consumption shown by saline and lithium groups in Fig. 1. In a final experiment we tested the hypothesis that emerged from the preceding experiments, that fluid consumption after lithium injection is controlled primarily by the interaction of the rat's exploratory response to a novel context and the rapid conditioning of an aversion to a novel fluid presented there.

2. Experiment 1

Our first experiment was intended to demonstrate that the pattern of consumption presented in Fig. 1 could be replicated with our present procedures. Rats were injected with either saline (NaCl) or lithium chloride (LiCl), and then were transported to a novel place where their ingestion of a novel sucrose solution was measured at 5-min intervals for the next 30 min. In so far as possible, employed the same procedures (e.g., used concentrations of lithium and sucrose) as those used by Symonds and Hall (2002).

2.1. Method

2.1.1. Subjects

The subjects in were 22 female albino Wistar-derived rats, bred in the animal colony of the Faculty of Education and Humanities of Ceuta (University of Granada). They were approximately 100 days old and had a mean weight of 236 g (range: 260–220 g) at the start of the experiment.

2.1.2. Apparatus

The rats were individually housed one week before the beginning of the experiment in a set of transparent plastic boxes measuring 43 cm × 27 cm × 19 cm, situated in a colony room that was maintained under a 12-h light/dark cycle (lights on at 8:00 a.m.) and at an ambient temperature of 23 °C. The top of the cage was made of wire mesh and the floor was covered by a layer of wood shavings. The rats had free access to food, and water, when available (see below), was presented through 350-ml plastic bottles equipped with a stainless steel ball-bearing-tipped-spouts. Fluid consumption was measured (to 0.5 g) by weighing these bottles.

The test was conducted in a novel context. This consisted of a separate experimental room dimly lit by a 50-W red bulb, and with speaker supplying background white noise at 50 dBA. This room housed a set of cages made of transparent plastic and having commercially obtained cat litter instead of wood shavings. They differed also from the home cages in size (48 cm × 38 cm × 21 cm).

2.1.3. Procedure

After a week in the individual home cages with ad libitum food and water, a schedule of water deprivation was introduced. The water bottles were removed overnight and, over the next two days access was limited to two 30-min sessions starting at 10:00 a.m. and 5:00 p.m. In order to allow the animals to become accustomed to the procedure to be used in the test session, on each of these 30-min drinking sessions the water bottles were removed every 5 min and replaced with an identical set of bottles.

The rats were randomly assigned to two groups of 11 for the test phase. The test was conducted at 10:00 a.m. The rats were removed from their home cages and given an intraperitoneal injection, either of LiCl (0.15 M, at 10 ml/kg of body weight) or of an equivalent dose of saline (NaCl). They were then transferred to the context cages. Bottles containing fluid, a 3.4% sucrose solution, were then immediately made available. The bottles were changed every 5 min during
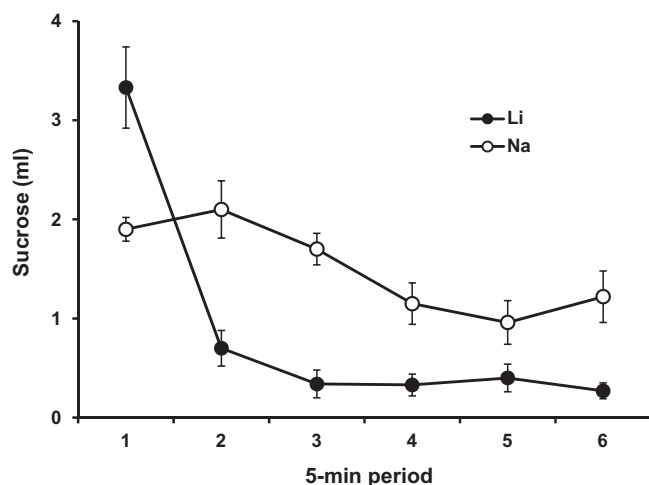
Fig. 2. Experiment 1. Group mean scores for sucrose ingestion over six consecutive 5-min intervals in a novel context for groups injected with lithium (Li) or saline (Na). Vertical bars represent the standard error of the mean.

Fig. 3. Experiment 2. Group mean scores for sucrose ingestion over six consecutive 5-min intervals in the home cage for groups injected with lithium (Li) or saline (Na). Vertical bars represent the standard error of the mean.

the 30-min session, allowing consumption in each 5-min period to be recorded. On average, the interval between the administration of the injection and the onset of the first test period was approximately 8 min. The procedures were approved by the University of Granada Ethics Committee for Animal Research, which agree with the NIH of the United States guidelines for the ethical treatment of animals, as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Results and discussion

Fig. 2 shows the group mean amounts of fluid consumed over successive 5-min periods of the 30-min test for rats given a novel test fluid in a new environment. The pattern closely resembles that of Fig. 1 from Symonds and Hall (2002). Overall, subjects injected with saline drank more than those treated with lithium but, during the first 5-min interval, this difference was reversed with subjects in the experimental group ingesting more than the controls. An analysis of variance (ANOVA) confirmed this description of the results. The analysis revealed a significant effects of group, $F(1, 20) = 12.44$, of the test intervals $F(5, 100) = 29.72$, and a significant interaction between these two variables $F(5, 100) = 14.76$. (A significance level of $p < .05$ was adopted here and throughout.) Separate analyses showed that groups differed significantly on each of the six 5-min periods, smallest $F(1, 20) = 4.65$. These results thus confirm the finding of Symonds and Hall (2002) that rats given a novel substance in a novel place, drink more during the very beginning of the test if they are treated with lithium, but that, thereafter, lithium induces an almost complete suppression of consumption. The remaining the experiments seek to determine the variables that operate to produce these effects.

3. Experiment 2

In this experiment we replicated the essentials features of Experiment 1, the only difference being that the test was conducted in the familiar environment of the home cage. We know from the work of Domjan (1977) that overall consumption of the novel sucrose solution is likely to be reduced in lithium-injected subjects; we do not know if the initial overconsumption will be found in these circumstances.
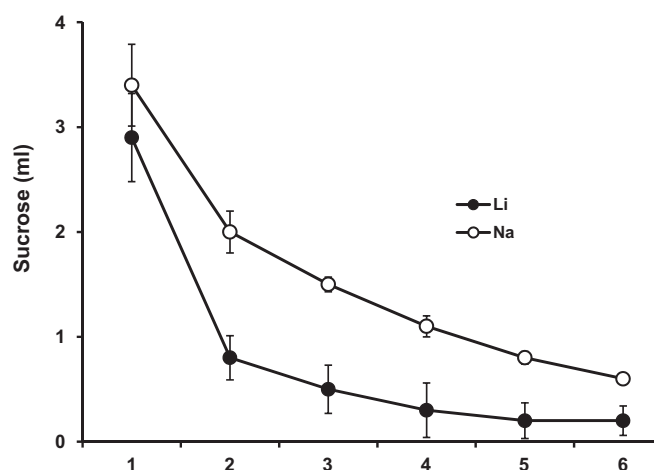
3.1. Method

The subjects were 20 female albino Wistar-derived from the same source as those used in Experiment 1. Ten were assigned to the lithium-treated groups and 10 to the saline group. They were 75 days old and had a mean weight of 210 g (range: 180–230 g) at the start of the experiment. The apparatus and procedure were as described for Experiment 1, differing only in that after the injection on the test day the rats were returned to their home cages. Given that it was not necessary to transport them to the context room, the interval between the administration of the injections and access to the sucrose was reduced to approximately 3 min.

3.2. Results and discussion

Fig. 3 shows for both groups the mean amounts of the novel sucrose solution consumed in the home cages over the six 5-min intervals of the test. The two groups showed the same pattern, drinking progressively less as the session continued. In addition, the lithium-treated rats drank consistently less than the control subjects. An ANOVA conducted on these data revealed a significant effect of group, $F(1, 18) = 34.10$, and of the test period, $F(5, 90) = 48.29$, but not of the interaction between these variables, $F(5, 90) = 1.28$.

The results of this experiment make clear that the apparent overdrinking by the lithium group seen in Experiment 1 (and by Hall and Symonds, 2002) depends on the test being given in a novel context. Furthermore, the effect of the nature of the context derives from its influence on the behavior of the control group. The lithium group showed the same pattern of consumption in the novel and familiar contexts – the apparent overdrinking was a consequence of the fact that the control subjects consumed rather little during the first test period when the test was given in a novel context. These results suggest that there is some factor that tends to suppress initial consumption in a novel context in control subjects but which fails to affect the behavior of rats injected with lithium. This last issue will be taken up later; the next experiment investigates possible sources of the pattern of behavior shown by control rats.

4. Experiment 3

One possibility is that, in the control condition at least, the novelty of the test context acts to potentiate the neophobic response

to the novel sucrose solution (Sjödén and Archer, 1981). An alternative (suggested, e.g., by Chance and Mead, 1955; Ennaceur et al., 2009) is that the lesser consumption of rats put into a new context might be a consequence of interference between the exploratory responses evoked by that context and the consummatory response. According to this idea, the suppressive effect produced by the context should not be dependent on the novelty of the fluid to be measured. Accordingly, in the present experiment we replicated the procedure of Experiment 1, testing lithium-injected and control rats in a novel context, but using a familiar substance (water) as the test fluid. If the initial low level of consumption shown by the control subjects in Experiment 1 was a consequence of interference from exploratory behavior, then the same effect should be evident in this experiment.

As a further test of the competing response notion we also manipulated the nature of the novel context used in the test. Honey et al. (1992, see also Barnett, 1963), argued that interference with consummatory behavior was more likely when the test cage was large (simply because exploration of such a cage would particularly likely to take the rat away from the drinking spout). To investigate this possibility we divided our two main groups into subgroups tested either in small or large novel cages. The experiment thus used a factorial (2×2) design. As in previous experiments, the rats were injected either with lithium (Li Groups) or with NaCl (Na Groups) prior to the test. Half the subjects in each group were tested in small cages (Li-Small and Na-Small Groups) while the rest were tested in the large boxes (Li-Big and Na-Big Groups).

In summary, if the initial reduction in consumption observed in control subjects in Experiment 1, was due to the interference between exploration and drinking, the same effect should be reproduced here with water as the test fluid. Additionally, according to the interference hypothesis, this reduction may be more marked in rats tested in big than in small cages. The notion that context novelty is effective because it enhances neophobia, on the other hand, has no reason to predict reduced initial consumption in either big or small cages.

4.1. Method

The subjects in Experiment 3 were 37 female rats from the same stock as was used in Experiment 2. They had a mean weight of 285 g (range: 270–300 g) and an age of 100 days at the start of the experiment. Two sets of cages, both different from the home cage, were available in the same room that had served as the novel context in Experiment 1. They were made of transparent plastic and contained commercially obtained cat litter instead of wood shavings. They differed in size: big (48 cm × 38 cm × 21 cm), small (43 cm × 27 cm × 19 cm), but were otherwise identical.

Subjects were placed in individual home cages with ad libitum food and water for a week, and then were subjected to a water deprivation regime. Over the next two days (or three days for some subjects, see below), access was limited to two (morning and evening) 30-min sessions. The test was conducted at 10:00 a.m. on the next day for subjects tested in the large cages and on the following day for those tested in the smaller cages. The rats were removed from their home cages and given an intraperitoneal injection, either of LiCl (0.15 M; 10 ml/kg) or of an equivalent dose of saline (NaCl). They were then transferred to the context cages and bottles containing tap water were then immediately made available. On the first day 10 rats were injected with lithium and nine with saline, before being transferred to the big cages in the experimental context. On the second day, equivalent groups (nine in each) were tested in the smaller cages. Other procedural details were as described for Experiment 1.
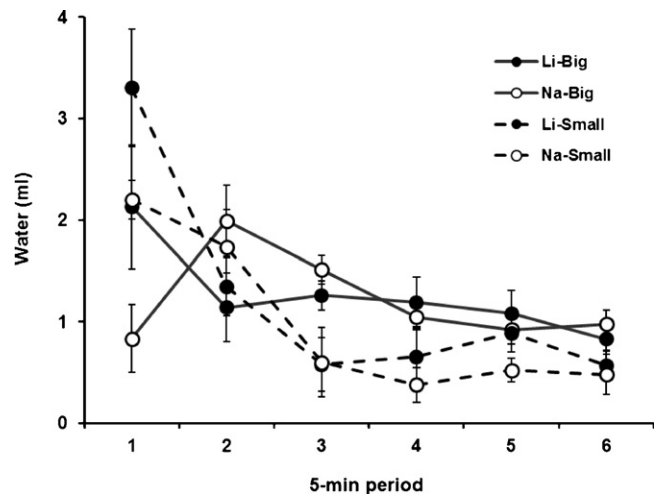

Fig. 4. Experiment 3. Group mean scores for water ingestion over six consecutive 5-min intervals for groups injected with lithium (Li) or saline (Na) in a novel Big or Small cage. Vertical bars represent the standard error of the mean.

4.2. Results

The mean amount of water consumed by each of the four groups over the six 5-min intervals of the test is shown in Fig. 4. It shows that both size of the test context and the type of injection given influenced ingestion over the course of the test. At the very beginning of the test, subjects injected with lithium drank more than those injected with saline. This pattern was reversed in the next test period. Thereafter, there was little difference between the lithium and saline groups. Additionally, differences emerged between subjects tested in large and those tested in small cages; consumption was greater in the initial period in the small cages, but fell in succeeding periods being somewhat lower than that seen in the large cages.

These data were subjected to an ANOVA, with cage size and injection type as between-subject variables and test interval as a within-subject variable. There was a significant main effect of interval, $F(5, 165) = 13.37$, but not of the size of the cage, $F(1, 33) = 3.01$, or of the injection, $F(1, 33) = 3.43$. The three-way interaction was not significant, $F < 1$, but there were significant interactions both between cage size and test interval, $F(5, 165) = 6.04$, and between kind of injection and test interval, $F(5, 165) = 4.04$.

These interactions were explored through analyses of simple effects. For the interaction between injection type and interval the analysis revealed that the injection factor was significant both in the first interval $F(1, 198) = 16.74$, and in the second $F(1, 198) = 4.79$, confirming that saline-injected animals drank less on the first interval and more in the second interval. These differences replicated those obtained in our first experiment and by Symonds and Hall (2002): subjects injected with saline drank less than those treated with lithium during the first 5 min, whereas during the next period the opposite pattern was found. Given that the initial diminution of consumption in the control groups occurred here with water as the test fluid, these results argue against the suggestion that the effect is a consequence of enhanced neophobia, but accord with the notion that exploration of the context (at least in control subjects) interferes with consumption early in the test. A parallel analysis of the interaction between cage size and test interval showed significant effects of cage size during the first test period $F(1, 198) = 18.71$, and also in the third, $F(1, 198) = 7.67$, and fourth, $F(1, 198) = 4.49$, intervals. As expected, then, consumption was initially less in the large than in the small cages. The differences present in later test periods presumably arise because subjects tested in the large cages

compensate for their initial reduced ingestion by increasing consumption in subsequent periods, when the novelty of the context has declined.

If we accept that control subjects, particularly those tested in the large cages, show reduced consumption in the first test period because they are exploring the novel context, it remains to explain why those injected with lithium are apparently largely immune to this effect. A possible answer comes from the work of Cappeliez and White (1981) who have suggested that lithium increases attention to salient stimuli and reduces the extent to which the animal is distracted by irrelevant stimuli. Given that the water bottle will be salient and relevant to a thirsty rat, this implies that the lithium treated rats would be more likely to drink from the bottle and less likely to explore the new environment. Support for this interpretation comes from Cappeliez and White's (1981) Experiment 2. This showed that presentation of a novel stimulus (a tone) to thirsty rats licking for water interrupted licking in animals previously injected with saline, but that this effect was reduced in animals injected with lithium.

5. Experiment 4

Our previous experiments have examined the case in which both test fluid and context were novel (Experiment 1) and those in which just one of these was novel (the fluid in Experiment 2, the context in Experiment 3). The present experiment looks at the remaining case, in which both fluid and context are familiar. This procedure will allow us to confirm, for our procedures, that an injection of lithium produces a suppression of consumption only when the test fluid is novel. It will also allow confirmation of the conclusion that the low level of consumption shown by control subjects in the initial test period is a consequence of interference from responses evoked by novel contextual cues. In the present experiment, with the test conducted in the home cage, no such interference should be obtained.

5.1. Method

The subjects were 19 rats from the same stock as those used in the previous experiments. They were 3 months old and had a mean weight of 285 g (range: 270–300 g) at the start of the experiment. Ten received an injection of LiCl, and nine an injection of NaCl before being tested with water in the home cage. Details of the procedure were as described for Experiment 2.

5.2. Results

The results of Experiment 4 for rats tested with a familiar fluid in a familiar context are shown in Fig. 5. Both groups drank more in the first test period than in later periods, showed a steady decline over the course of the test, and there were no differences between them. An ANOVA showed only a significant effect of the test periods, $F(5, 85) = 114.82$ (other $Fs < 1$). These results thus confirm that the suppression of consumption produced by lithium will be found only when the fluid offered after the injection is novel. Additionally, they confirm that the context played a critical role in the differing consumption showed by the two groups in the first test period in Experiments 1 and 3. The reduced level of consumption shown by control subjects in the first period is only observed when the test context is novel, consistent with the suggestion that this effect depends on competition between exploratory and consummatory responses.
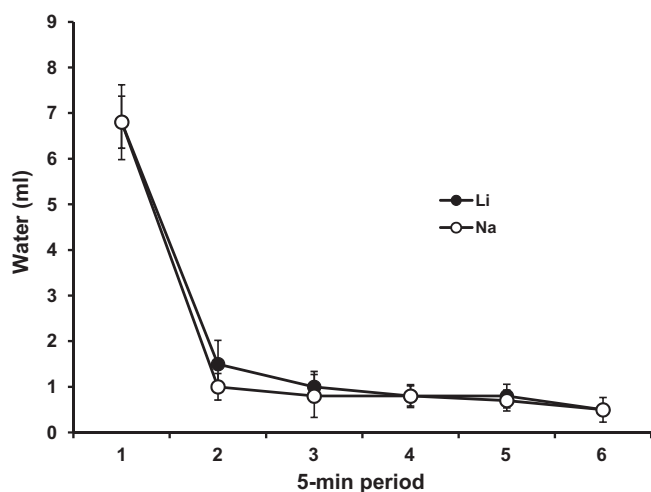

Fig. 5. Experiment 4. Group mean scores for water ingestion over six consecutive 5-min intervals in the home cage for groups injected with lithium (Li) or saline (Na). Vertical bars represent the standard error of the mean.

6. Experiment 5

Our results so far show that the difference between lithium and control subjects in the initial period of a test (in which the latter consume less than the former) depends on the novelty of the context. When the context is familiar (or has become so over the course of the test), the reverse result is obtained – but only if the test fluid is novel. Reduced consumption in lithium-treated animals was observed when the test fluid was sucrose, not when it was water. It remains to explain this latter effect.

One possibility is that this reduced consumption reflects a poisoning-induced enhancement of neophobia. As we have noted, this interpretation is challenged by the fact that consumption is normal early in the test session, when the fluid is quite novel (although it might be argued that this indicates simply that a lithium injection takes a few minutes to have its effect). An alternative possibility is that this suppression reflects not an unconditioned effect, but the rapid acquisition of conditioned aversion – that experience of the novel taste early in the session in the presence of lithium-induced illness, establishes an aversion to the taste that suppresses later consumption. Support for this possibility comes from a study by Spector et al. (1988) who gave rats a series of intraoral infusions of sucrose every 5 min, for 30 min, immediately after an injection of lithium. They scored ingestive (e.g., tongue protrusion) and aversive (e.g., gaping) responses to the infusion, and found that ingestive responses were frequent at the start of the test (the lithium-injected rats showing the same levels of responding as controls), but that thereafter the poisoned rats showed a steady decline in ingestive responding and a steady increase in the frequency with which they emitted aversive responses (see also Cross-Mellor et al., 2004, for a similar effect). Spector et al. concluded that their procedure had established a conditioned taste aversion, a conclusion supported by the fact that the aversive responding was maintained in a test given several days later.

In order to test this idea, in the present experiment we gave rats access to sucrose for 5 min in the novel context after an injection either of lithium or of saline. If the results of Experiment 1 are replicated, we can expect the lithium-treated animals to drink the sucrose readily during this period; indeed more readily than the control subjects. The rats were then removed from the context and allowed two days to recover from the effects of the injection. After this period, consumption of sucrose was tested (in the home cage). If the suppressed consumption seen in the later periods of the test in
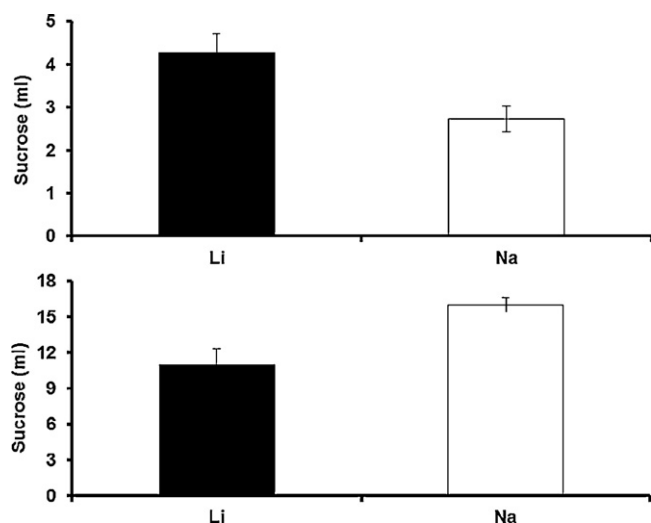
Fig. 6. Experiment 5. Top panel: Group mean scores for sucrose ingestion in the 5 min immediately following an injection of lithium (Li) or saline (Na). Lower panel: Group mean scores for sucrose ingestion in a 30-min test given 48 h after the injections. Vertical bars represent the standard error of the mean.

Experiment 1 is the consequence of acquisition of an aversion during the first period, then lithium-injected rats can be expected to show reduced consumption on this test, given that a conditioned aversion will persist over the interval.[1] If, on the other hand, the later test results of Experiment 1 reflect the enhancement of a neophobic response produced by increasing illness over the course of the test, then no such reduced consumption should be evident in the delayed test.

6.1. Method

The subjects were 22 female rats obtained from the same stock as those used in Experiment 1 having a mean free weight of 250 g (range: 275–230). As in the previous experiments, the procedure started by restricting the water access in the home cages over three days. Given that it was not necessary to interchange the bottles on the sucrose tests, water access in the deprivation phase was uninterrupted for the 30 min of both sessions, morning and evening. On the fourth day the rats received injections of lithium or saline at 10:00 a.m., and were immediately transported to the experimental room where they had access to the sucrose solution for 5 min. They were their were returned to the home cages and received the water recovery session in the evening. Water deprivation was maintained over the next two days. The final test was conducted on the third post-injection day in the morning, and consisted of uninterrupted access to sucrose for 30 min. Other procedural details were as described for Experiment 1.

6.2. Results and discussion

The top panel of Fig. 6 shows the amount of sucrose (group means) consumed during the 5 min that followed the administration of lithium or of saline. As in Experiment 1, the lithium-injected group drank more than the control group during this period. A

one-way ANOVA confirmed that the difference was significant, $F(1, 20) = 8.09$. When tested 48 h later, however, the pattern of consumption was reversed; the lower panel of Fig. 6 shows that on this test the lithium group consumed less than the control group, a difference that was again significant, $F(1, 20) = 10.71$. These results thus support the suggestion that although they will initially drink it readily, consumption of the sucrose solution in rats injected with lithium will establish a conditioned aversion that remains evident two days later when the direct effect of the injection have subsided. The rapid acquisition of a conditioned aversion could thus be responsible for the suppression of consumption seen in the later stages of the test by the lithium group of Experiment 1.

7. General discussion

The results of these experiments can be summarized simply. Our first experiment replicated the effect found by Symonds and Hall (2002), confirming that rats injected with lithium and given access to a novel fluid in a novel context drank more than control rats in the first 5-min period of the test, but less in subsequent periods. Our new experiments show that the elements of this biphasic pattern are separately controlled by the novelty of the test fluid and the novelty of the test context. When just the test fluid was novel (Experiments 2) a general suppression of consumption was obtained in animals given lithium. When the context alone was novel (Experiment 3), the first phase of the pattern was seen, with lithium-treated rats drinking more than controls. And when neither fluid nor context was novel (Experiment 4), there was no difference between the groups. Finally, Experiment 5 replicated the initial sucrose overingestion in rats injected with lithium and exposed to a new place, but the subsequent rejection was observed on this occasion under different circumstances: 48 h later and in the home cages.

To summarize the explanation that has emerged for this pattern of findings, it will be convenient to deal separately with the two phases of the response, beginning with that shown in the first 5-min period. As we have already noted, antidotal drinking does not provide an adequate explanation of the enhanced consumption shown by lithium-treated rats in our first experiment and in the experiment by Symonds and Hall (2002). (This is not to say that the antidotal thirst does not apply to the effects produced by other emetic substances; just to say that our new data seem to confirm the inadequacy of this explanation for the Symonds and Hall results.) If extra consumption in the first 5 min was indeed a response serving to mitigate the oncoming illness, it might be expected to occur just as readily in a familiar as in a novel context. But no such effect was observed in Experiment 4, which differed from Experiment 3 in which the effect was obtained, only in that the test context was familiar.

This last observation prompted consideration of the possibility that the effect depends on the responses evoked by the novel context. Following Chance and Mead (1955) we suggested that rats will drink rather little when they first encounter a new place because they are engaged in exploration. Some support for this account comes from the analysis of differences found in Experiment 3 between the groups tested in the large and small cages. Exploring a large cage is particularly likely to take the rats away from the drinking tube and, accordingly, the initial consumption observed in Experiment 3 might be expected especially low in animals in this condition. The mean total score over the first 5-min period for animals tested in the large cages was 2.9 ml (2.1 ml for subjects given lithium and 0.8 ml for saline-injected controls); the equivalent score for those tested in the small cages was 5.5 ml (3.3 ml for subjects injected with lithium and 2.2 ml for saline groups).

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[1] Given that the emetic effects of the lithium are almost immediate, this manipulation has some features of a "backward conditioning" procedure. However, it must be noted that, as the gastric illness is fairly long-lasting (about 30 min for the dose we use) this procedure means that the ingestion of the flavor is not only preceded, but also followed, by the gastric illness, as in any other ordinary forward conditioning paradigm. Demonstrations of aversion conditioning using this sort of procedure have been supplied, e.g., by Boland (1973), and Franchina (1989).

We conclude that the low level of consumption shown by the control subjects at the start of the test in Experiments 1 and 3 (and by the equivalent subjects in the study of Symonds and Hall, 2002) reflects the effect of competing responses evoked by the novel context. It remained to explain why this effect should be less marked in the animals injected with lithium. Our answer, based on the work of Cappeliez and White (1981), was that one effect of lithium is to increase attention to salient stimuli and reduces the extent to which the animal is distracted by irrelevant stimuli. For a thirsty rat, the water bottle will be salient, making lithium-treated rats more likely to drink from the bottle and less likely to explore the new environment. However, it should be noted that the supposed reduction in the exploratory behavior in the experimental group could be also due to a lithium-induced diminution in general activity (Batson, 1983). And we should acknowledge that there is currently rather little evidence to confirm the notion that lithium can modify attentional focus of the rat; it would be useful if further research confirmed the generality of the idea, for example, by testing if an initial overdrinking can be replicated using other fluids than sucrose or water.

We turn now to the suppression of consumption shown by lithium treated animals, evident in the later phases of the test in our Experiment 1 and in the experiment by Symonds and Hall (2002) (when, presumably, the effects produced by initial exploration of the novel context are no longer powerful), and also throughout the test (carried out in a familiar context) in Experiment 2. The key feature of these experiments (that distinguishes them from Experiment 3 and 4) is that the test fluid was the novel sucrose solution. This finding is generally consistent with Domjan's (1977) suggestion that lithium produces an enhanced neophobic reaction, but it still remains a problem for this account that the lithium-injected rats in the experiment by Symonds and Hall (2002), as well as in our first experiment, drank sucrose quite readily in the first 5-min period of the test. According to our interpretation, these rats drink more than the controls because they are focused on the drinking tube rather than exploring the novel context. But if neophobia is enhanced by the lithium treatment, a tendency to focus on the tube, and thus on the novel fluid it contains, might be expected to evoke a particularly strong neophobic reaction to the sucrose that is being experienced for the very first time at the start of the test.

The alternative possibility is that the suppression of sucrose consumption is the consequence of the rapid conditioning of an aversion – that the experience of a novel taste in the presence of a developing illness is enough to generate a conditioned aversion to that flavor during the course of the test itself (Spector et al., 1988). This idea accounts for results found by Symonds and Hall (2002) as well as in our Experiments 1 and 5. The fact that the test fluid needs to be novel for this to occur would then be taken to indicate, not neophobia, but the absence of a latent inhibition effect.

The implication is that the suppressed consumption of a novel fluid shown by rats injected with lithium is not, as we had originally supposed, an instance of a UR; rather it is the consequence of the formation of a conditioned response (CR). Specification of the nature of the UR to lithium has theoretical and practical significance. Context aversion conditioning, in which exposure to a particular context is paired with lithium, has been examined by studies in which it is demonstrated that rats will reduce their consumption of a novel fluid when it is presented in the conditioned context (e.g., Best et al., 1984; Rodríguez et al., 2000). It has been assumed that this response reflects the ability of the context to evoke the state of nausea as a CR, and this procedure has been offered as a model for the phenomenon of anticipatory nausea, in which patients undergoing chemotherapy start to experience nausea in response merely to the contextual cues supplied by the clinic (e.g., Stockhorst et al., 1998; Hall and Symonds, 2006). The validity of the animal model depends on the assumption that the conditioned context indeed evokes nausea as a CR. Part of the evidence taken to support this assumption came from the fact that the response evoked by the conditioned context (suppression of consumption) appeared to match what was taken to be the UR directly evoked by an injection of lithium (Symonds and Hall, 2002; Hall and Symonds, 2006). Our present results (which suggest that the suppressed consumption evoked by lithium itself may be a CR) undermine this argument.

This analysis does not prove that context conditioning does not endow the context with the power to evoke nausea; rather, it means that we will need to use measures other than suppression of consumption to prove the point. Limebeer et al. (2006) have used a taste reactivity test of the sort used by Spector et al. (1988) and have shown that a context previously associated with lithium will evoke aversive responses (such as gaping) like those seen in response to a flavor that has been paired with lithium. Further research could return to the issue of the relation between the UR evoked by lithium and the CR it supports as a US. The clearest UR evidenced by the experiments reported here is the tendency of lithium injected rats to attend to the drinking tube rather than explore a novel context; new experiments could investigate if a context previously paired with lithium induces an equivalent change in attentional focus in rats.

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